

Re: linker peptide in two-domain fusion protein

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Subject: Re: linker peptide in two-domain fusion protein
From: Simon Brocklehurst (smb18@mbfs.bio.cam.ac.uk)
Date: Mon 26 Jul 1993 - 11:32:36 BST

- **Next message:** Ian McDonald: "[Hydrogen Bond Calculator](#)"
- **Previous message:** FJvanderWal: "[needed: random mutation+doped oligo's](#)"
- **In reply to:** GIRISH SAHNI, SCIENTIST, IMTECH SECTOR 39-A, CHANDIGARH 160014, INDIA: "[linker peptide in two-domain fusion protein](#)"
- **Reply:** Simon Brocklehurst: "[Re: linker peptide in two-domain fusion protein](#)"

girish@imtech.ernet.in (GIRISH SAHNI, SCIENTIST, IMTECH SECTOR 39-A, CHANDIGARH 160014, INDIA) writes:

>Dear Freind, Hi there:

>I need some suggestions for designing a linker sequence between two
 >independently folded protein domains in a fusion (gene) construct.
 >Perhaps we would have to try a few candidate sequences (3-10 AA long)
 >with varying degrees of flexibility/rigidity, since we cannot predict
 >a priori what the effect may be on unwanted aggregation or steric
 >hindrance associated with the final hybrid protein. Sources where I
 >might ponder these aspects will be welcome, as would actual AA sequences
 >of possible linker peptides. Thank you
 >Girish Sahni IMTECH, India
 >Email: "girish@imtech.ernet.in"

There are probably two kinds of sequence that you might want to explore.

- 1) Sequences made up of Ala and Pro. By varying the number of Ala-Pro pairs you can modulate the flexibility of the linker.
- 2) Sequences made up of charged amino acid residues e.g. mixing Glu and Lys. It is more difficult to rationilise how flexibility can be modulated by sequence variation in this case.

Both these types of sequence are common in long (30-40 aa) linkers joining independently folded domains in multifunctional polypeptides.

A good reference to start with is the review by Richard Perham
 "Domains, motifs and linkers in 2-oxo acid dehydrogenase multienzyme complexes: a paradigm in the design of a multifunctional protein"
 Biochemistry 30, 8501-8512

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Dept. of Biochemistry
University of Cambridge
Tennis Court Road
Cambridge
UK

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- Antigen recognition February 1994 to February 1996
- Antigen recognition February 1996

Biophysical studies of T-cell receptors and their ligands. *Review article*

Daved H Fremont, William A Rees and Haruo Kozono

Current Opinion in Immunology 1996, 8: 93-100.

Outline

- Abstract
 - Introduction
 - Production of soluble proteins
 - Thermodynamic and kinetic measurements
 - Structural studies
 - Conclusions
 - Acknowledgements
 - References and recommended reading
 - Copyright
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Abstract

Recently developed methodologies for the production of the soluble extracellular domains of

☒ α β TCRs have allowed several biophysical characterizations. The thermodynamic and kinetic parameters associated with specific ligand interactions between the TCR and MHC-peptide complexes, as well as superantigens, are now being established. Crystallographic studies of isolated

TCR fragments have yielded the structures of a V ☒ α domain and the two extracellular domains of a β -chain. These investigations are beginning to allow a new visualization of antigen

recognition and T-cell activation processes.



Introduction

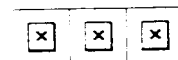
Spurred on by the availability of soluble components, biophysical studies of the molecules at the heart of cellular immunology are coming to fruition. Although there are many cell surface glycoproteins on the outer membranes of T cells, antigen specificity is dictated by two sets of

polypeptides: either TCR ☒ alpha chain and β chain, or ☒ gamma and ☒ delta chain. Each chain is composed of a variable (V) and constant (C) immunoglobulin-like domain, a single transmembrane region and a short cytoplasmic tail. These chains come together to form a disulfide bonded heterodimer, which is intimately associated with a cluster of transmembrane proteins collectively

known as CD3 (☒ epsilon, ☒ delta, ☒ gamma) and the TCR ☒ zeta -chain. The two V domains of the ☒ alpha β TCR control the specificity of the protein for peptides bound to MHC molecules, which are expressed on the surface of antigen-presenting cells (APCs).

Interactions of TCRs with MHC ligands can have several effects on T cells, depending on the developmental maturity of the cell and the quality of the adhesion. The prevailing hypothesis is that immature T cells in the thymus are positively selected for maturation if they recognize cells expressing self-MHC complexes with a low binding affinity. If the molecular recognition event involves an affinity above a certain threshold, negative selection ensues, leading to apoptosis. For mature T cells in the periphery, the strong and weak interactions may induce reverse effects (i.e. a strong interaction leads to activation, whereas a weak one leads to anergy). Experimental evidence argues that, in addition to intrinsic affinity, these outcomes are also directly related to the density of ligand on the APC, a combined effect termed either efficacy [1] or avidity [2]. A different model argues that only the ligands that induce specific conformational changes in the TCR may be able to activate T cells [3•] [4]. Thus, the role of affinity and structure in T-cell selection and activation is of utmost importance for successful immune responses and is a subject of great interest today.

In this review, we discuss the various efforts that have been made to obtain soluble TCR fragments (Table 1), the thermodynamic and kinetic data that have been obtained with these molecules (Table 2) and the status of structural studies of the TCR (Fig. 1).



Production of soluble proteins

A variety of methods have been developed for the production of the soluble, extracellular domains of TCRs (Table 1). In contrast to MHC proteins [5] [6] [7] [8], the successful manufacture of functional heterodimeric TCRs has been the exception rather than the rule. Heterodimer assembly is generally

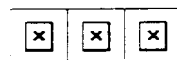
inefficient *in vitro* [9], and the secretion of ☒ alpha β TCRs from cells that harbor properly paired chains lacking transmembrane and cytoplasmic sequences is idiosyncratic [9]. Strategies to avoid the vagaries of heterodimer association have been developed and involve either the synthesis of fusion proteins, or the production of single-chain molecules.

In the fusion proteins, the transmembrane and cytoplasmic regions of each chain have been replaced with immunoglobulin constant domains [10] [11], leucine zippers [12•], CD3 γ -chain domains [13] [14•] or sequences from lipid-linked proteins [15]. Soluble TCR is typically liberated from the secreted or membrane-associated fusion proteins by enzymatic cleavage. Another method that has been pursued with varying degrees of success has been the construction of linked V

domains, using either a peptide linker from the carboxyl terminus of the V α domain to the amino terminus of the V β domain [16] [17] [18•] [19] [20], or a disulfide bond engineered into the V α -V β interface [21•]. The fraction of the recovered material that is properly folded and functional in physiologically-relevant ligand-binding assays has not been well established. The

expression and purification of a functional, single chain molecule composed of V α V β C β domains connected by flexible peptide linkers has recently been reported [18•]. The requirement for C β in the proper folding of this construct may be related to the observation that the V β and C β domains have extensive interactions with each other in the β chain crystal structure (see below). The

dispensable nature of the C α domain has parallels in that the α 3 domain from MHC class I molecules is also not required for the proper folding of the other extracellular domains [22]. Crystallization conditions have been described for two different single chain fragments [23] [24], but to date no such report has been made for a TCR $\alpha\beta$ heterodimer.



Thermodynamic and kinetic measurements

The initial experiments designed to explore the affinity of TCR for its ligands employed solubilized MHC proteins binding to cell surface TCR, or vice-versa. The first estimate of an equilibrium

dissociation constant (K_D) was 10^{-7} μ M, based on the decreased production of IL-2 in an alloreactive T cell that was blocked from its recognition of target cells by an engineered soluble MHC class I molecule [25]. In a competition assay between soluble class II proteins and a labeled Fab fragment of an anti-TCR antibody, Matsui *et al.* [15] found a much weaker affinity (K_D

10^{-5} μ M) for the peptide-specific recognition of MHC by TCR. They also found no difference in affinity in the presence or absence of CD4. In a similar set of experiments, Sykulev *et al.* [26•] have shown that the dissociation constants of various soluble class I MHC-peptide complexes bound to a T-cell clone can vary by a 1000-fold

(10^{-4} - 10^{-7} μ M), with a good correlation between affinity and activity. Antibody blockage of CD8 function appeared to have no effect on their measurements. More recent experiments by Sykulev *et al.* [27•] comparing two different TCRs and their ligand binding affinities have provided evidence to support the view that TCRs have a higher affinity for allogeneic MHC-peptide complexes compared to syngeneic complexes, potentially related to the lack of negative selection operative on TCRs recognizing the former. In the reverse experiment, Weber *et al.* [11] inhibited T-

cell activation by a soluble, chimeric TCR enabling a K_D of 10^{-5} μ M. These experiments suffer from series of caveats, the most serious of which is that the intrinsic affinity of TCR for MHC is not measured directly and may be affected by the presence of other cell-surface molecules. Nevertheless, taken together, these results indicate that the affinity of the TCR for MHC is rather weak, and is comparable to several other protein-protein interactions that are known to mediate

intercellular interactions [28].

As T-cell selection and activation may well be a matter of the 'on and off' rates of the TCR with its ligands, not simply the dissociation constants, recent studies have focused on kinetic measurements. The technique of choice has been surface plasmon resonance (SPR) using solubilized components and the BIAcore Biosensor System. The advantage of this method is that it allows for the measurement of the fast 'on and off' rates that are typical of low affinity interactions. Possible disadvantages of this method, however, include the steps necessary to attach one component to the acidic surface of the BIAcore chip (i.e. steps that have the potential to modify the nature of the protein). The first SPR experiments showed that the kinetic dissociation rate of a class I peptide complex ($L^d/p2C$) from the alloreactive 2C TCR is quite rapid, with a half-time of dissociation of

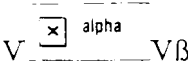
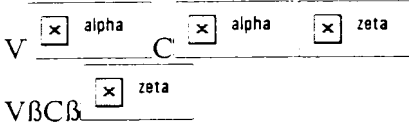

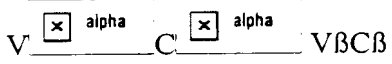

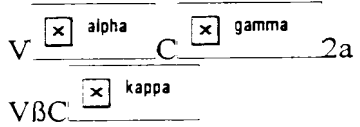


$t_{1/2} = \frac{\ln 2}{k_{\text{thinsp}}} = \frac{\ln 2}{k_{\text{thinsp}}}$ 27 seconds [29]. The kinetic parameters of this same receptor-ligand pair differ from estimates from cellular inhibition, which argue for an eightfold slower dissociation rate and a fourfold slower on-rate [26]. SPR experiments by Matsui *et al.* [30] on 2B4 TCR interacting with I-E^k-peptide complexes indicate off-rates similar to those observed for the 2C TCR (i.e. $t_{1/2}$

$t_{1/2} = \frac{\ln 2}{k_{\text{thinsp}}} = \frac{\ln 2}{k_{\text{thinsp}}}$ 2-12 seconds), but 100-fold slower on-rates. Matsui *et al.* argue that MHC-peptide complexes that are weaker stimulators of T-cell activation are primarily distinguished by their faster off-rates. This is in contrast to the observations of Sykulev *et al.* [26], who report a strong correlation of 2C cytotoxic T lymphocyte (CTL) activity with dissociation constants of the MHC ligand. One simple argument would be that TCRs associate more tightly with MHC class I versus class II complexes, an idea that is consistent with data indicating that there is a lower number of TCRs on CD8⁺ T cells. A different argument that has been proposed, however, is that as the 2B4 affinities are generally significantly weaker, and potentially at the demarcation of some critical threshold value, the off-rates assume a dominance not necessarily observed in the allogeneic 2C experiments [30]. Taken together, these affinity and kinetic measurements are consistent with the basic tenants of the avidity model of T-cell selection and activation.

The more recent data on the 2C TCR from Al-Ramadi *et al.* [31] muddy the water a bit. Using a combination of assays, including SPR, to study a series of variant peptides, these authors conclude that the apparent TCR-MHC affinity does not consistently correlate with T-cell activation, potentially showing the limitations of the BIAcore instrument in the measurement of slow on-rate/fast off-rate interactions. In contrast to previous reports, these experiments uncovered a role for the CD8 co-receptor in the rescue of MHC ligands that are poor T-cell activators, a role that is apparently not required for high affinity ligands, so long as they are at high surface density. These results are consistent with the experiments of Luescher *et al.* [32] which suggest that CD8 not only adheres to cell surface MHC class I proteins, but also coordinates with the TCR in a trimolecular complex.

Table 1. Summary of production of soluble TCR domains.

TCR	Expression system	Domains expressed	β TCR formed?	Tricks
2B4	Refolded from <i>E. coli</i> inclusion bodies	V α V β	Yes	Disulfide bo engineered f V α 42
2B4	Secreted from	V α C α VBC β	Yes	Secreted wit chain carboh

2B4	CHO cells Refolded from <i>E.coli</i> inclusion bodies		Yes	sites remove 29 amino ac linker betwe variable dom
2B4	Cleaved from RBL-2H3 cells		Yes	 cha transmembra cytoplasmic attached to e thrombin rel soluble TCR
2B4	Cleaved from CHO cells		Yes	GPI linkages chains; solub released by e cleavage; po of  heterodimer
2B4	Sp 2/0 cell lysates		No	V regions lin C domains
2C	Cleaved from BWS-197 thymomas		Yes	Both chains GPI anchor
2C	Refolded from <i>E.</i> <i>coli</i> inclusion bodies			25 amino ac linker betwe domains

JM22 T-cell clone	Refolded from <i>E. coli</i> inclusion bodies	$V \begin{array}{ c } \hline \alpha \\ \hline \end{array} V\beta$		Peptide linker V domains: questionable properly refolded material
DO-11.10 (1.3)	Secreted from Baculovirus-infected Sf9s cells	$V \begin{array}{ c } \hline \alpha \\ \hline \end{array} C \begin{array}{ c } \hline \alpha \\ \hline \end{array} V\beta C\beta$	Yes	Heterodimer predominant
N15	Baculovirus High 5 cells	$V \begin{array}{ c } \hline \alpha \\ \hline \end{array} C \begin{array}{ c } \hline \alpha \\ \hline \end{array} V\beta C\beta$	Yes	Basic leucine zipper on $\begin{array}{ c } \hline \alpha \\ \hline \end{array}$ improve heterodimer formation
Hy.2H9	Cleaved from Bw5147 β -cells	$\begin{array}{ c } \hline \alpha \\ \hline \end{array} - V \begin{array}{ c } \hline \alpha \\ \hline \end{array} V\beta C\beta$		15 amino acids from $V \begin{array}{ c } \hline \alpha \\ \hline \end{array}$ GPI linkage
KB5-C20	Secreted from melanoma cells	$V \begin{array}{ c } \hline \alpha \\ \hline \end{array} C \begin{array}{ c } \hline \alpha \\ \hline \end{array} C \begin{array}{ c } \hline \kappa \\ \hline \end{array}$ $V\beta C\beta C \begin{array}{ c } \hline \kappa \\ \hline \end{array}$	Yes	Ig light chain attached to $\begin{array}{ c } \hline \alpha \\ \hline \end{array}$ an questionable heterodimer
Jurkat cells	Secreted from Sp 2/0 cells	$V \begin{array}{ c } \hline \alpha \\ \hline \end{array} C \begin{array}{ c } \hline \alpha \\ \hline \end{array} V\beta C\beta$	No	Ig C domain to each chain
1934.4	Secreted from <i>E. coli</i>	$V \begin{array}{ c } \hline \alpha \\ \hline \end{array} V\beta$		Peptide linker V domains: questionable properly folded material Ig C regions
PH28	J558L and NSO myelomas	$V \begin{array}{ c } \hline \alpha \\ \hline \end{array} V\beta$	No	$\begin{array}{ c } \hline \alpha \\ \hline \end{array}$ an V regions: only $V \begin{array}{ c } \hline \alpha \\ \hline \end{array} C$ chimeras secreted
14.3d	J558L myelomas	$V \begin{array}{ c } \hline \alpha \\ \hline \end{array} C \begin{array}{ c } \hline \alpha \\ \hline \end{array} IC \begin{array}{ c } \hline \kappa \\ \hline \end{array}$		V and first C of each TCR linked to C β

		VBCB1Cβ			
					exon of <input checked="" type="checkbox"/> light chain 23 amino ac from V <input checked="" type="checkbox"/> ^a Vβ; certain hydrophobic predicted to surface were to increase s Peptide linke V domains; tagged; ques yields of pro refolded mat
RFL3.8 (human)	Secreted from <i>E. coli</i>	V <input checked="" type="checkbox"/> alpha Vβ			
1934.4	Secreted from <i>E. coli</i> and Ni ²⁺ -NTA chromatography	V <input checked="" type="checkbox"/> alpha Vβ			
T195/BW (mouse <input checked="" type="checkbox"/> gamma <input checked="" type="checkbox"/> delta)	Secreted from COS cells	V <input checked="" type="checkbox"/> alpha C <input checked="" type="checkbox"/> alpha V <input checked="" type="checkbox"/> delta C <input checked="" type="checkbox"/> delta	Yes		Ig C domain to each chain
G9 (human <input checked="" type="checkbox"/> gamma <input checked="" type="checkbox"/> delta)	Secreted from COS cells	V <input checked="" type="checkbox"/> alpha C <input checked="" type="checkbox"/> alpha V <input checked="" type="checkbox"/> delta C <input checked="" type="checkbox"/> delta	Yes		Introduction translation te codons upstr sequences en membrane s regions in ea
F23.1 (anti-Vβ8), 1B2 (anti-clonotypic 2C TCR), KJ16 (anti-Vβ8), A2B4 (ANTI-2B4 <input checked="" type="checkbox"/> alpha chain binding), H57-597 (anti-Cβ), KJ25 (anti-Vβ3), C1 (anti-Vβ17, dependent on <input checked="" type="checkbox"/> alpha β pairing), F10/56 (anti-clonotypic for T195 TCR), TiV <input checked="" type="checkbox"/> delta 2 (anti-V <input checked="" type="checkbox"/> delta 2), B121 (anti-C <input checked="" type="checkbox"/> gamma).					
Return to table reference 1, 2					



Structural studies

In the past decade, X-ray crystallography has contributed significantly to our understanding of the molecules involved in the cellular immune response. Indeed, the first direct evidence that class I MHC molecules bound antigenic peptides came from the pioneering crystallographic work of Bjorkman *et al.* [33]. Crystal structures have been determined for single peptide complexes of several class I MHC proteins [34] [35] [36] [37] [38], class II proteins [39] [40] [41] and a non-classical MHC [42]. In addition, the structure of HLA-DR1 complexed with two different bacterial superantigens, staphylococcal enterotoxin B (SEB) [43] and toxic shock syndrome toxin-1 (TSST-1) [44], have been determined. There has also been substantial progress on the structure of the T-cell co-receptors CD4 [44] [45] [46] and CD8 [47]. More recently, the crystal structure of the tandem SH2 domains of Zap-70 in complex with a peptide from the cytoplasmic tail of the TCR ☒ delta

chain has been determined [48].

Table 2. Summary of thermodynamic and kinetic measurements using soluble TCRs.

TCR	Ligands	Peptide sequence	K_D (M)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	Method
HTB157.7	K^b (Q10b hybrid)	Heterogeneous	1.0 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-7}			50% inh producti
HTB157.7	pK^b 163-174	NA	1.0 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-4}			50% inh producti
2C	$L^d/p2Ca$	LSPFPFDL*	5.0 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-7}	1.1 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^4	5.5 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-3}	Kinetics of 1B2 F TCR
2C	$L^d/p2Ca$ -A5	LSPFAFDL	6.3 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-5}			50% inh Fab' bind
2C	$L^d/p2Ca$ -A3	LSAFPFDL	5.0 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-5}			50% inh Fab' bind
2C	$L^d/p2Ca$ -A8	LSPFPFDA	5.9 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-7}			50% inh Fab' bind
2C	$L^d/SL9$	SPFPFDLLL	7.1 <input type="checkbox"/> thinsp <input type="checkbox"/> times			50% inh Fab' bind

		<input checked="" type="checkbox"/> thinsp		
		10^{-5}		
		3.3 <input checked="" type="checkbox"/> thinsp		
2C	K ^b /p2Ca LSPFPFDL	<input checked="" type="checkbox"/> times		50% inh Fab' bind
		<input checked="" type="checkbox"/> thinsp		
		10^{-4}		
		6.7 <input checked="" type="checkbox"/> thinsp	5.3 <input checked="" type="checkbox"/> thinsp	3.1 <input checked="" type="checkbox"/> thinsp
2C	L ^d /QL9 QLSPSPDL	<input checked="" type="checkbox"/> times	<input checked="" type="checkbox"/> times	<input checked="" type="checkbox"/> times
		<input checked="" type="checkbox"/> thinsp	<input checked="" type="checkbox"/> thinsp	<input checked="" type="checkbox"/> thinsp
		10^{-8}	10^4	10^{-3}
		6.7 <input checked="" type="checkbox"/> thinsp	2.2 <input checked="" type="checkbox"/> thinsp	2.1 <input checked="" type="checkbox"/> thinsp
4G3	K ^b /pOV8 SIINFEKL	<input checked="" type="checkbox"/> times	<input checked="" type="checkbox"/> times	<input checked="" type="checkbox"/> times
		<input checked="" type="checkbox"/> thinsp	<input checked="" type="checkbox"/> thinsp	<input checked="" type="checkbox"/> thinsp
		10^{-7}	10^5	10^{-1}
		1.2 <input checked="" type="checkbox"/> thinsp	2.1 <input checked="" type="checkbox"/> thinsp	2.6 <input checked="" type="checkbox"/> thinsp
2C	L ^d /p2Ca LSPFPFDL	<input checked="" type="checkbox"/> times	<input checked="" type="checkbox"/> times	<input checked="" type="checkbox"/> times
		<input checked="" type="checkbox"/> thinsp	<input checked="" type="checkbox"/> thinsp	<input checked="" type="checkbox"/> thinsp
		10^{-7}	10^5	10^{-2}
		2.7 <input checked="" type="checkbox"/> thinsp		
2C	L ^d /p2Ca LSPFPFDL	<input checked="" type="checkbox"/> times		BIACore
		<input checked="" type="checkbox"/> thinsp		
		10^{-6}		
		2.9 <input checked="" type="checkbox"/> thinsp		
2C	L ^d /p2Ca-Y4 LSPYPFDL	<input checked="" type="checkbox"/> times		BIACore
		<input checked="" type="checkbox"/> thinsp		
		10^{-6}		
		7.4 <input checked="" type="checkbox"/> thinsp		
2C	L ^d /p2Ca-A1 ASPFPFDL	<input checked="" type="checkbox"/> times		BIACore
		<input checked="" type="checkbox"/> thinsp		
		10^{-6}		

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CPUS 91.12630 - 12631, 1991

2B4	I-E ^k /MCC- ANERADLIAYLKQASK T102S	<div> <div>thinsp</div> <div>10⁻⁴</div> <div>1.0</div> <div>times</div> <div>thinsp</div> </div>	0.1-0.3	50% inh
HA1.7	SEB	<div> <div>thinsp</div> <div>8.0</div> <div>times</div> <div>thinsp</div> <div>10⁻⁷</div> </div> <div> <div>thinsp</div> <div>1.3</div> <div>times</div> <div>thinsp</div> <div>10⁴</div> </div> <div> <div>thinsp</div> <div>1.1</div> <div>times</div> <div>thinsp</div> <div>10⁻²</div> </div>		BIACore
14.3d β	SEC1	<div> <div>thinsp</div> <div>2.0</div> <div>times</div> <div>thinsp</div> <div>10⁻⁵</div> </div>		BIACore
14.3d β	SEC2	<div> <div>thinsp</div> <div>7.9</div> <div>times</div> <div>thinsp</div> <div>10⁻⁶</div> </div>		BIACore
14.3d β	SEC3	<div> <div>thinsp</div> <div>9.2</div> <div>times</div> <div>thinsp</div> <div>10⁻⁶</div> </div>		BIACore
14.3d β	SEB	<div> <div>thinsp</div> <div>1.4</div> <div>times</div> <div>thinsp</div> <div>10⁻⁴</div> </div>		BIACore
14.3d β	SPEA	<div> <div>thinsp</div> <div>6.2</div> <div>times</div> <div>thinsp</div> <div>10⁻⁶</div> </div>		BIACore

14.3d β	SEC1	2.5 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-6}	Sedimen equilibri
14.3d β	SEC2	2.3 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-6}	Sedimen equilibri
14.3d β	SEC3	8.6 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-7}	Sedimen equilibri
14.3d β	SEB	7.0 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-5}	Sedimen equilibri
14.3d β	SPEA	2.1 <input type="checkbox"/> thinsp <input type="checkbox"/> times <input type="checkbox"/> thinsp 10^{-6}	Sedimen equilibri

*Single-letter code for amino acids.

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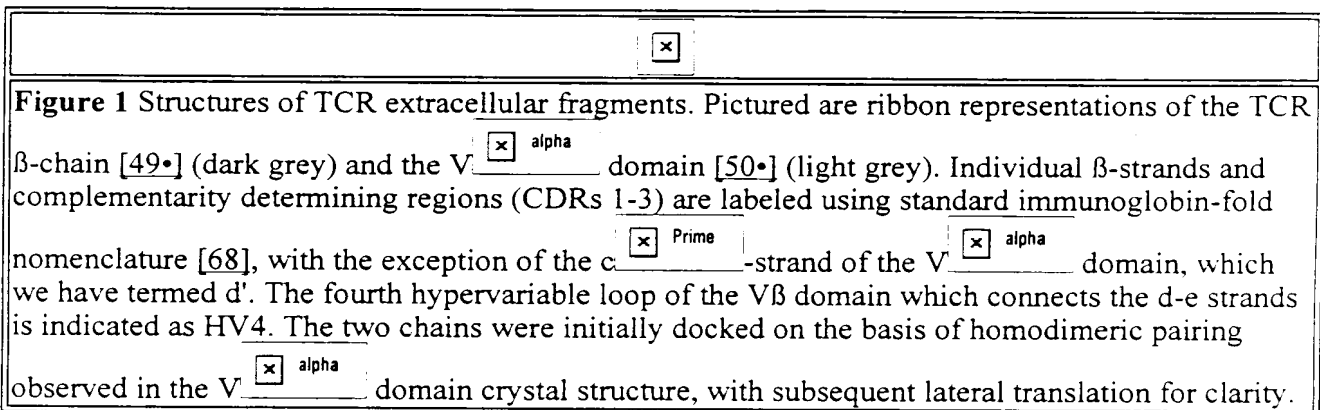
Although this year has seen the visualization of fragments of both chains, structural characterization of the ☐ α β TCR has proven far more elusive, [49•] [50•] (Fig. 1). Using a construct of the extracellular TCR β chain, in which most of the possible *N*-linked glycosylation sites were removed by mutagenesis, Bentley *et al.* [49•] has established the immunoglobulin domain structural similarities for both murine $V\beta$ 8.2 and $C\beta$, the former closely resembling the V_L domain of antibodies and the latter generally resembling antibody C domains. Unlike antibody C domains, however, the structure of the $C\beta$ domain contains a large loop connecting the last two strands of the

sandwich, a region predicted to lie away from the ☐ α -chain in the heterodimer and which could interact with adjacent proteins in the membrane (i.e. CD3). Analysis of the complementarity determining regions (CDRs) of the $V\beta$ domain has indicated that the CDR1 and CDR2 loops are likely to be fairly restricted in size and conformation, consistent with predictions that their predominant role is in binding to the flanking MHC helices, whereas the hypervariable CDR3 localizes over the antigenic peptide [51] [52] [53]. More recent data on the binding of a panel of

TCRs to mutant MHC class I molecules argues that recognition is skewed towards residues at the carboxy-terminal ends of the flanking helices (H2-K^b residues 70-90 and 155-174), predicting that the TCR sits parallel to the underlying β -strands of the peptide-binding platform [54].

The functional significance of the isolated, unglycosylated β chain is supported by SPR and sedimentation equilibrium binding measurements with superantigens, which indicate fast association and dissociation kinetics [55•]. The hypervariable region of V β 8.2, anticipated to interact directly with superantigen HV4 [56], is predominantly exposed to solvent and adopts a conformation quite similar to the analogous region of a V_L domain. One surprise of the β chain structure is an extensive

interaction ($> \text{thinsp } 800 \text{ \AA}^2$) between the V and C domains not previously seen in antibody Fab structures (200-350 \AA^2 , depending on the elbow angle between the V and C domains). Bentley *et al.* [49•] argue that if this interaction were maintained in a TCR α β heterodimer, then the elbow angle would be relatively fixed, with the implication that signal transduction could be facilitated by a ligand-dependent conformational change in a rigid TCR. Crystal structures of a free and MHC-bound α β TCR are clearly necessary to address this possibility.



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The crystal structure of murine TCR V α 4.2 domain produced in bacteria has very recently been reported [50•]. The V α domain is unique among immunoglobulin family V domains in that the fifth strand (conventionally termed c' α Prime), which follows sequentially the CDR2 loop, forms antiparallel β -sheet interactions with the d-strand in the a-b-e-d sheet rather than the c'-strand in the c'-c-f-g sheet (Fig. 1). The suggestion has been made that this strand be termed d' (WA Hendrickson, personal communication). In contrast to the crystallized TCR β -chain, the V α domain is a homodimer both in solution and in the crystal, with a V α -V α association highly reminiscent of the V_L-V_H pairings of antibodies. Fields *et al.* [50•] suggest that V α -V β heterodimers may associate just as the V α homodimers do, although docking of the two structures reveals a steric clash of the V β CRD3 loop with the α chain (Fig. 1). This may be a reflection of the fact that these two chains do not naturally associate, but another plausible explanation is that the V β CRD3 loop conformation of Bentley *et al.* [49•] is artifactual due

to the absence of an associated ☒ alpha chain in the crystal structure.

In addition to the typical V-V domain pairing observed in the V ☒ alpha structure, Fields *et al.* [50•] suggest that there is good evidence in the crystal packing for a dimer of TCRs formed as a result of the unusual V ☒ alpha d' (c ☒ Prime) strand swap described above. They argue in support of a dimer of heterodimers, in which most of the contact surface is composed of residues from V ☒ alpha, by noting that the V ☒ alpha and V β glycosylation sites would fall on the outside of the proposed tetramer. They also state that the CDRs of such a dimer of heterodimers would have a planar disposition, with an alignment that could facilitate the interaction of the two V ☒ alpha V β combinations with a surface bearing a dimer of MHC ligands. It is unclear whether the proposed dimer of heterodimers is consistent with the rigid V β -C β interaction noted by Bentley *et al.* [49•].

The proposed V ☒ alpha-V β dimer of heterodimers has a superficial structural complementarity to the dimer of HLA-DR heterodimers observed in multiple crystal forms [57] [58]. If the TCR were to exist as a dimer as proposed by Fields *et al.* [50•], and if it were to interact with a HLA-DR dimer,

then the CDR3 of V ☒ alpha would lie over the amino-terminal end of the antigenic peptide in the MHC groove, and the CDR3 of V β would lie over the carboxy-terminal end of the same peptide. This orientation is consistent with the results of some mutagenesis experiments [59]; however, the notion that this is the correct orientation of the TCR over MHC, or that there is an invariant orientation, is controversial [60] [61]. Moreover, in order for the TCR and MHC to dock in this fashion, a large conformational change would be required in one or both dimers so that both the TCRs could engage both MHC ligands, as the peptides in the HLA-DR dimer are markedly non-planar. There is also the problem that it is unlikely that both MHC molecules of a pre-formed cell-surface dimer would be bound by the same peptide [62] [63]. Further complicating matters is the observed (and potentially artifactual) superdimers of I-E^k bound with covalent peptides [41]. The peptides in this oligomer are planar as well as anti-parallel, but the docking topology of the Fields *et al.* [50•] TCR dimer is even worse, as only the peptide and β chain could be contacted.

Oligomers of MHC class I have not been observed in the multiple crystal structures that have been determined, but a plausible dimer model supported by mutational data has been proposed based on

interactions of one CD8 ☒ alpha ☒ alpha homodimer and two class I molecules [64]; however, this complex also seems inconsistent with the TCR dimer model, as the class I molecules are not immediately adjacent to one another. Although evidence exists that low affinity homophilic interactions can give rise to co-operative superstructures implicated in cell-cell adhesion [65], the data for TCR and MHC doing so are, at present, minimal. Some degree of multimerization of the TCR-CD3 complex seems likely, although other explanations are available for how low affinity interactions can lead to signalling [66]. Typically, receptors involved with signal transduction function as oligomers at the cell surface after ligation [67]. There is some doubt, however, about the biological significance of homotypic TCR and MHC interactions formed prior to their engagement with one another.



Conclusions

There are currently several methods available for the production of soluble and functional TCRs and MHC proteins. Interactions of TCRs with their ligands are characterized by low affinity and fast kinetic off rates. Completely absent from the physical picture is our knowledge of the stoichiometry of the components involved during selection and activation. The role of co-receptors in mediating productive signalling is also far from established. The crystal structures of two isolated TCR

fragments (V ☐ alpha and V β -CB) have recently been determined. These structures have established the immunoglobulin-fold similarities for the TCR domains, and revealed novel variations. These studies serve as an appetizer for the main course of our understanding of the roles played by receptor structure and oligomerization in T-cell stimulation.



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
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
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
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linker peptide in two-domain fusion protein

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Subject: linker peptide in two-domain fusion protein

From: GIRISH SAHNI, SCIENTIST, IMTECH SECTOR 39-A, CHANDIGARH 160014, INDIA
(girish@imtech.ernet.in)

Date: Fri 23 Jul 1993 - 06:58:17 BST

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- **Previous message:** [Keith O. Elliston: "UDP Glucose Binding Motif"](#)
- **Next in thread:** [Simon Brocklehurst: "Re: linker peptide in two-domain fusion protein"](#)

Dear Freind, Hi there:

I need some suggestions for designing a linker sequence between two independently folded protein domains in a fusion (gene) construct. Perhaps we would have to try a few candidate sequences (3-10 AA long) with varying degrees of flexibility/rigidity, since we cannot predict a priori what the effect may be on unwanted aggregation or steric hindrance associated with the final hybrid protein. Sources where I might ponder these aspects will be welcome, as would actual AA sequences of possible linker peptides. Thank you

Girish Sahni IMTECH, India
Email: "girish@imtech.ernet.in"

-
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